The *Steinernema glaseri* group and its morphological characteristics

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SUMMARY

Among the 31 *Steinernema* species described so far and presently recognised, ten are considered here as members of the *S. glaseri* group, named after the first species described in this group. The taxonomic status of several members of this group of obviously closely related species appears questionable and the distinction of species by certain morphological features doubtful. This applies, in particular, to morphometric characters which often exhibit a wide range of variation. Three of the species (*S. glaseri, S. arenarium, S. longicaudum*) have been recorded for several countries or even continents, the other species are known from their type localities and type countries only.

The *S. glaseri* group is mainly characterised and distinguished from other species and species groups in the genus *Steinernema* by morphological characters of the third-stage infective juveniles: Mean body length > 800 µm; excretory pore located posterior to mid-pharynx region; lateral fields with nine distinct lines (= eight ridges), which are evenly spaced and developed; lip region broad, rounded or slightly flattened and in general (almost) continuous with body contour; hyaline tail portion mostly shorter than half tail length. In males the excretory pore is also behind mid-pharynx (often at level of terminal bulb) and a tail mucron is in general absent. The shapes of spicules and gubernaculum are rather variable among the species of the group. An interesting feature and possibly a unique character within the genus *Steinernema* is the obvious absence of a distinct bacterial vesicle in the third-stage infective juveniles, which often exhibit a wide intestinal lumen.

INTRODUCTION

Steinernema species with long third-stage infective juveniles are generally considered as a distinct group within the genus, and molecular analyses have supported evidence of such a species group and close relationships of the species (Hominick *et al*., 2001; Stock *et al*., 2001b; Reid, 2003). With increasing number of species described in the genus *Steinernema* morphological distinction of a "*glaseri*-group", based mainly on body length of the juveniles, is becoming more difficult. Moreover, due to demonstrated high variation in body length, identification of individual juveniles as members of the group is often impossible.

In this contribution the described species considered as members of the *S. glaseri* group are briefly discussed and, based mainly on morphological characteristics of the infective juveniles, a morphological "diagnosis" of the group is proposed. For this, published data on morphometrics and other morphological features were used, supplemented by own studies. Third-stage juveniles were available from *S. glaseri* isolates of various origin, *S. arenarium* from Russia and other European countries, *S. cubanum, S. longicaudum, S. karii*, *S. loci*, *S. thanhi* and *glaseri*-like or unidentified populations from Italy, Morocco, Palestine, China, Vietnam, New Zealand, South Africa and USA. Glycerin mounts of many other *Steinernema* species were available for comparison. Studies on morphology and species identity of isolates of the *S. glaseri* group from Europe are presented separately (Sturhan & Mráček, 2003). A brief characterisation of the *S. glaseri* group has already been given recently (Sturhan & Mráček, 2001).

SPECIES IN THE *S. GLASERI* GROUP AND THEIR DISTRIBUTION

Among the 31 *Steinernema* species described so far and presently recognised, I consider the following ten as members of the *S. glaseri* group, named after the first described species in this group and presented here in the sequence of their description:

S. glaseri (Steiner, 1929) Wouts, Mráček, Gerdin & Bedding, 1982

S. arenarium (Artyukhovsky, 1967) Wouts, Mráček, Gerdin & Bedding, 1982

syn. *S. anomali* (Kozodoi, 1984) Curran, 1989

S. caudatum Xu, Wang & Li, 1991

S. longicaudum Shen & Wang, 1992

S. cubanum Mráček, Hernández & Boëmare, 1994

S. puertoricense Román & Figueroa, 1994

S. karii Waturu, Hunt & Reid, 1997

S. ohioense Lucskai & Klein in Lucskai, 1999

S. loci Phan, Nguyen & Moens, 2001

S. thanhi Phan, Nguyen & Moens, 2001

Molecular analyses, in which six of the species have been included, showed that the *S. glaseri* group forms a well distinguished group within the genus *Steinernema* (Stock *et al*., 2001b; Reid, 2003).

Steinernema glaseri was described from New Jersey, USA, and mostly considered as a subtropical or even tropical species (Poinar, 1986; Poinar & Kozodoi, 1988; Kung *et al*., 1990). It has been reported also for other states in the USA, from Brazil, Argentine, China, South Korea, the Azores and Spain (Poinar, 1978; Pizano *et al*., 1985; de Doucet, 1990; Li & Wang, 1989; Wang *et al*., 1991; Stock *et al*., 1997; Rosa *et al*., 2000; de Doucet & Gabarra, 1994). The worldwide occurrence of this species is not surprising, because it had been introduced for biological control of insects in the 30ies to Hawaii, Australia, New Zealand and distributed "throughout to world" (Poinar, 1978), which may have resulted in establishment of the species in various regions of the world. *Steinernema arenarium* is known from Central Russia and it appears that most European *glaseri*-like populations are conspecific with this species (see Sturhan & Mráček, 2003). *Steinernema longicaudum*, described from China, has subsequently been isolated from Australia (Hominick *et al*., 1996), California, USA (Stock *et al*., 1999) and South Korea (Stock *et al*., 2001a), the Californian populations eventually being not conspecific with *S. longicaudum* (Stock *et al*., 2001b). The other species of the *S. glaseri* group are apparently known so far only from the type localities and countries mentioned in the original descriptions: *S. cubanum* from Cuba, *S. puertoricense* from Puerto Rico, *S. ohioense* from USA, *S. karii* from Kenya, *S. caudatum* from China, *S. loci* and *S. thanhi* from Vietnam. Most of the species appear to prefer subtropical or tropical regions, with probably only *S. arenarium* being more cold-adapted.

NOTES ON INDIVIDUAL SPECIES

Steinernema glaseri. Steiner (1929) described this species from larvae of the Japanese beetle (*Popillia japonica*) in New Jersey, USA, without providing measurements and a detailed description of the juveniles. Poinar (1978) based the redescription on material obtained from larvae of the scarab beetle *Strigoderma aboricola* in North Carolina, added measurements of a

strain from Florida later (Poinar, 1986), and data of both populations were subsequently combined (Poinar & Kozodoi, 1988). Data on the hyaline tail portion of the infective juveniles are lacking so far. I measured (n=12): tail length = 80 (75-85) µm, hyaline tail portion = 38 (34-40) µm = 47 (44-48) % of the total tail length. The suspected presence of *Steinernema* species in the USA close to *S. glaseri* (*S. ohioense?* strain NC 513? others?) may require studies on the species identity of populations from the subtropical regions with those of New Jersey, where the winters are very cold and the summers rather short and cool. This would include also reconsideration of the *S. glaseri* DNA genebank accessions!

Steinernema arenarium. In morphometrics of infective juveniles and adults this species is very similar to *S. glaseri.* In various keys (Nguyen & Smart, 1992; Hominick *et al*., 1997; Lucskai, 1999) the distance of the excretory pore from the anterior end in the infective juveniles, D % of the males and the spicule length were used as characters differentiating both species. Data from the redescription of *S. arenarium* show that such differences do not exist. The distance of the excretory pore from the anterior end (EP) can be calculated from the new data presented as 98 µm (against 102 µm in *S. glaseri* infective juveniles), and D % of males is 78-93 and the spicule length 76 µm (against 70-91 and 77 µm, respectively, in *S. glaseri*). Nguyen and Smart (1995) reported lower EP measurements for *S. arenarium (S. anomali*) juveniles (means: 85-98 µm) than for *S. glaseri* (means: 104-117 µm), but the total body length was also less than in *S. glaseri*. Poinar and Kozodoi (1988) considered differences in the tip of the spicules as the only consistent morphological characters that could be used to separate the adults of both species, but from the redescription of *S. arenarium* (Artyukhovsky *et al*., 1997) and my own observations it appears doubtful, if such differences do actually exist. *Steinernema arenarium* could be distinguished from *S. glaseri* in disc-electrophoresis pattern and in RFLP profiles (Poinar & Kozodoi, 1988; Artyukhovsky *et al.*, 1997; and others). In hybridization experiments by Poinar and Kozodoi (1988) both species did not interbreed.

Steinernema caudatum. The dimensions given for this species are in the range of those of *S. glaseri*; the distance of the excretory pore behind the anterior end in the infective juveniles (82 µm) and D % (53) are closer to that of *S. arenarium*. The shape of the spicules is most similar to *S. arenarium*, in both species with blunt tip. The shape of the female tail tip, which is used by Lucskai (1999) as a diagnostic character, is similar to that of *S. glaseri* and *S. arenarium*. The lateral field of the infective juveniles has (also) eight longitudinal ridges (Xu *et al*., 1991). Since the original description presented for *S. caudatum* does not allow distinction from *S. glaseri* and

S. arenarium, the species status of *S. caudatum* remains doubtful. Further studies are required, in particular molecular analyses and interbreeding experiments.

Steinernema longicaudum. This species was redescribed recently based on a study of specimens from the type isolate from China and two other isolates recovered from South Korea and the USA (Stock *et al*., 2001a); the drawing presented of the anterior end of the infective juvenile showing an anterior position of the excretory pore is incorrect, as is the scale given for the light microscopy image of the tail. *S. longicaudum* differs from *S. glaseri* and *S. arenarium* mainly in longer tail of the infective juvenile with a mean of 95 µm (against a maximum mean of 82 µm in *S. glaseri* and *S. arenarium*. I measured 90 (70-101) µm with a hyaline portion of 51 (46-55) % (n = 25) in specimens from cultures originating from China. Part of other differential morphometrics presented in the redescription are incorrect or not complete; no comparison was made with the similar species *S. puertoricense*, which has an identical tail length of the infective juveniles (mean: 94 µm). The shapes of spicules and gubernaculum can be used to differentiate the two species (Nguyen & Smart, 1997). The first generation female is characterised by a serratulate projection on the tail. The infective juveniles have a lateral field with nine equally spaced longitudinal lines (confirmed by my own light microscopical observations). The presence of a bacterial pouch is mentioned (Stock *et al*., 2001a); I observed no distinct vesicle but a wide anterior part of the intestine containing the symbiotic bacteria. The deirids are situated at pharyngeal bulb level. There is contradictory information on the species identity of populations from California. According to Stock *et al*. (2001a) a Californian isolate could interbreed with Chinese and Korean isolates, and DNA studies confirmed identity of all isolates as *S. longicaudum*. On the other hand molecular studies with the same isolates (same morphometrics given) by Stock *et al.* (2001b) "showed substantial sequence divergence" which "indicate that these morphologically similar isolates of *S. longicaudum* are not conspecific".

Steinernema cubanum. Distinguished from *S. glaseri, S. arenarium* and *S. longicaudum* by its comparatively short conoid tail (= high ratio c), short spicules and gubernaculum. Interbreeding with *S. glaseri* and *S. arenarium* (*S. anomali*) failed (Mráček *et al.*, 1994). I obtained the following morphometrics of infective juveniles (n = 10): L = 1125 (975-1220) µm, D % = 66 (62-74), tail = 63 (54-72) µm, hyaline tail portion = 45 (40-49) %, c = 18 (16-19).

Steinernema puertoricense. Similar in particular to *S. longicaudum*, but shapes of spicules and gubernaculum different (Nguyen & Smart, 1997; Adams & Nguyen, 2002). According to the

original description lateral fields of the infective juveniles with six incisures, but after Stock *et al*. (2001b) eight ridges (= nine incisures) present. There is no information on the length of the hyaline tail portion. *Steinernema puertoricense* did not hybridize with *S. cubanum* (Román & Figueroa, 1996).

Steinernema karii. This species is morphologically similar to the preceding species, but the body of the infective juveniles is shorter. From *S. glaseri, S. arenarium* and *S. cubanum* it can be readily distinguished in RFLP profiles (Waturu *et al*., 1997), from *S. longicaudum* in shorter tail of the infective juveniles. I observed nine equally spaced incisures in the lateral field of the juveniles from the type strain (eight incisures reported in the original description).

Steinernema ohioense. This species is imperfectly described by Lucskai (1999) in an identification key to entomopathogenic nematodes; morphometrics of infective juveniles were given, but no information on other morphological details (Lucskai, 1999). There are no data on males, except that the spicules have a notched tip, and for females it is mentioned only that the vulva is "symmetric". Figures and data on type locality, type specimens etc. are lacking. Despite inadequate description the species has to be accepted as valid according the International Rules of Zoological Nomenclature. *Steinernema ohioense* cannot be distinguished morphologically from *S. glaseri*; the distinguishing characters ratio E = 1.31 against E = 1.42 and "symmetric vulva" against "asymmetric vulva" must be considered as being insignificant. In males of a culture considered as *S. ohioense* I observed a small, 0.5-3 µm long tail mucron in most specimens, the excretory pore situated behind the middle of the pharynx and spicules of 61 µm length (chord).

Steinernema loci. This species is distinguished from most of the above mentioned species in morphometrics but close to *S. karii*, also in the shapes of male tail, spicules and gubernaculum (Phan *et al*., 2001). The differences to *S. karii* infective juveniles in the position of the excretory pore (80 µm *vs* 74 µm) and in pharynx length (141 µm *vs* 134 µm) appear insignificant and to be related to differences in total body length. The mentioned differences in spicule length and in the ratio SW (spicule length/cloacal body width) may be partly based on the way of measuring the spicule length (curved median line or chord). *S. karii* had not been included in the RFLP studies mentioned in the original description and interbreeding experiments were not conducted.

Steinernema thanhi. According to the original description by Phan *et al.* (2001) similar in morphometrics and male characters to *S. loci* and *S. karii*, but distinguished from *S. loci* in number and position of male genital papillae and RFLP pattern, from *S. karii* in shorter tail length of the infective-stage juveniles, higher ratio SW and shorter spicules in the males (spicules were measured along curved median line in *S. karii;* cp. notes under *S. loci*).

Of the ten species mentioned above, *S. ohioense* and *S. caudatum* have to be considered as doubtful and also *S. loci* and *S. thanhi* require more studies, in particular hybridisation experiments and extended molecular analyses. The description of more species placed in the *S. glaseri* group is expected. Populations which could not be assigned to any of the presently known species, have been found in several countries.

MORPHOLOGICAL CHARACTERISTICS OF THE *S. GLASERI* GROUP

Third-stage infective juveniles and males exhibit the most important diagnostic morphological characters in the genus *Steinernema*. A selection of characters commonly used for distinguishing species and considered here as essential for diagnosing the *S. glaseri* group are compiled in Table 1. In this table the range of means is given, followed by the total range of individual morphometrics (in brackets), with data taken from various publications and from my own studies added for *S. glaseri, S. arenarium, S. cubanum, S. ohioense, S. longicaudum, S. karii*, also for *S. kraussei* and *S. feltiae*. The species are arranged in descending order of body length and the ten members of the *S. glaseri* group are followed by the longest other species in the genus *Steinernema*.

This compilation of morphometrics in Table 1 shows a wide range in body length, in particular, for the better studied species. The actual expected range may be even wider. For instance, Itou (2000) gave a mean length of 906 µm for a Korean isolate of *S. glaseri* and Kakouli-Duarte & Hague (1999) a mean of only 581 µm for "small infective juveniles" developed in *Otiorhynchus sulcatus*. Nguyen and Smart (1995) have shown that morphometrics are considerably influenced by age of the culture and methods of cultivation. It is also a well-known fact that fixation and the kind of the fixative used have an influence on dimensions of nematodes, in particular, on body length. The morphometrics compiled on Table 1 were obtained from both fresh and unfixed specimens, as well as from specimens killed with different fixatives and from specimens transferred to permanent mounts in anhydrous glycerin; moreover, in many publications the

"treatment" of the specimens measured has not been mentioned. All these observations are indicating that minor differences in measurements should be used with caution. There is no distinction of the *S. glaseri* group possible from the other *Steinernema* species by body length of the infective-stage juveniles, but individual measurements observed so far are only rarely less than 800 µm, and the lowest mean of the presently known *S. glaseri* members is 850 µm (Table 1). The body length of >1000 µm, which is commonly used in keys to identify *S. glaseri* and related species (Poinar, 1990; Nguyen & Smart, 1996; 1997; Hominick *et al*., 1997; Lucskai, 1999; and others), can no longer be used to characterise the *S. glaseri* group.

The cephalic region is rather broad in the infective juveniles, rounded and mostly flattened anteriorly, continuous with body contour or at most slightly offset by a weak depression (Fig. 1). Its appearance is different in most other *Steinernema* species (narrower, angular, more offset, with horn-like projections etc.). The position of the excretory pore is consistently posterior to mid-pharynx (Tab. 1), while in other *Steinernema* species it is variable and mostly more anterior. Deirids and hemizonid are generally situated in the region of the pharyngeal bulb or slightly behind, in other *Steinernema* species mostly anterior to the bulb.

The lateral fields of the *glaseri* group infective juveniles, which are often very wide, are marked by nine equally developed lines (= eight ridges) arranged at even distances in midbody region (Fig. 3, A-C). In the redescription of *S. glaseri* (Poinar, 1978) and in the original description of *S. puertoricense* an incorrect number of six incisures had been given, in the original description of *S. karii* a number of eight. Several other *Steinernema* species have similarly arranged nine lines in the lateral field (e.g., *S. weiseri* and the undescribed *S.* "spec. B" from Germany), others (Fig. 3, D-F) have the submarginal incisures less developed (*S. feltiae, S. bicornutum, S. carpocapsae*) or a lower number of lines (*S. kraussei*, *S. kushidai*, *S. neocurtille*, *S. affine*).

The tail of the infective juveniles is straight or slightly curved ventrad and short to elongate conoid with a pointed or finely rounded tip, relatively wide at base (c' = tail length : anal body width = mostly 2-4), the ratio c >10, and the hyaline tail portion covers mostly less than half tail length (Fig. 2). The phasmids are distinct, situated mostly slightly anterior to mid-tail and shifted to the ventral side of the lateral field (same as probably in all other *Steinernema* species).

A remarkable feature, which appears to be a unique character within the genus *Steinernema*, is the obvious absence of a distinct bacterial vesicle with distinct wall (Fig. 4). The anterior resion

of the intestine containing the symbiotic bacteria is generally wide, with its anterior part often slightly offset; distinct walls are absent (Fig. 4). A wide intestinal lumen is commonly visible, generally well developed in the anterior region of the intestine, but occasionally extending up to the rectum. We observed such structures in infective-stage juveniles of *S. glaseri of different isolates*, *S. arenarium* populations of various origin, in *S. longicaudum*, *S. loci*, *S. thanhi*, the NC 513 isolate and in unidentified *S. glaseri*-like specimens from China, New Zealand and South Africa. We consider the lack of a distinct bacterial vesicle as a characteristic of the entire *S. glaseri* group, but more studies are required. The visibility of these characters depends, however, on nutritional condition, age, fixation etc. of the specimens studied.

In males of the members of *S. glaseri* group the excretory pore is also behind the mid-pharynx region, often at the level of the terminal bulb (Tab. 1). A tail mucron appears to be generally absent (Hominick et al., 1997; Adams & Nguyen, 2002), but occasinally a small mucron may be developed (observed in *S. arenarium*, *S. cubanum*, *S. ohioense*). In many other *Steinernema* species a well-developed mucron is present. The shapes of spicules and gubernaculum are rather variable amongst the species of the *S. glaseri* group.

The important diagnostic morphological characters of the *S. glaseri* group are summarized in brief: *Infective juveniles*. Body length >800 µm; D % >50; 9 lateral lines (8 ridges) in lateral field, evenly spaced and developed; lip region broad, (flatly) rounded, (almost) continuous; no distinct bacterial vesicle, lumen of intestine often wide; hyaline tail portion mostly <50 % of total tail length. *Males*. Tail mucron absent; D % >50 (-100).

ACKNOWLEDGEMENTS

For kindly providing nematode material I thank my colleagues B. Briscoe, R.-U. Ehlers, F. García del Pino, N. Iraki, I. Kramer, A.J. Meyer, A. Peters, K.L. Phan, A. Reid, N. Simões, P. Smits, S. Spiridonov, O. Triggiani and, in particular, Zdenék Mráček for fruitful cooperation.

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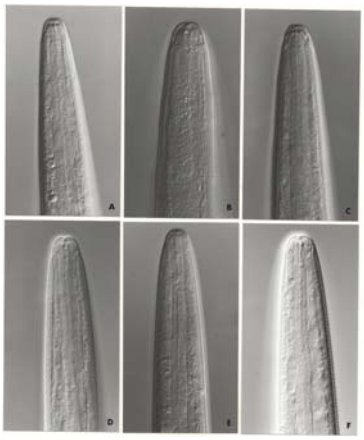

Fig. 1. Anterior ends of *Steinernema* third-stage infective juveniles. A: *S. arenarium* (Russia), B: *S. glaseri* (USA); C: *S. "glaseri"* NC 513 (USA); D: *S. cubanum* (Cuba); E: *S. karii* (Kenya); F: *S. longicaudum* (China).

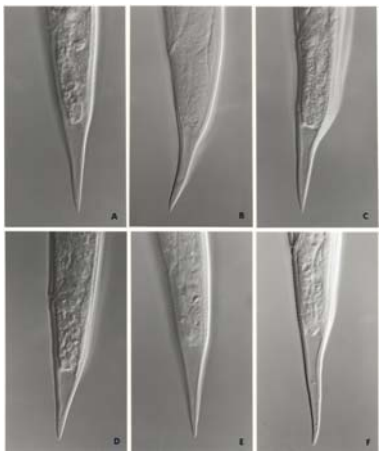

Fig. 2. Tails of *Steinernema* third-stage infective juveniles. A: *S. arenarium* (Russia); B: *S. glaseri* (USA); C: *S. "glaseri"* NC 513 (USA); D: *S. cubanum* (Cuba); E: *S. karii* (Kenya); F: *S. longicaudum* (China).

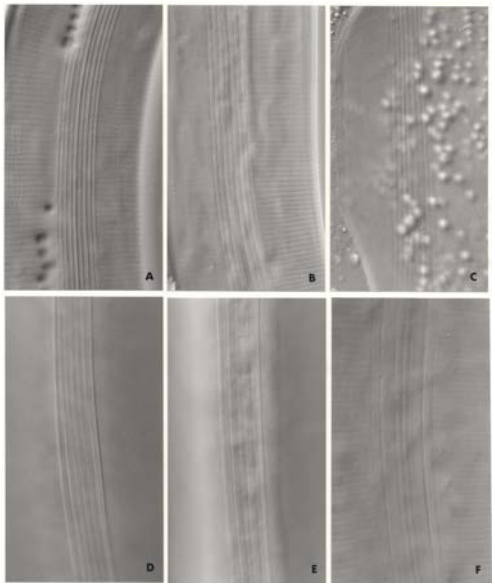

Fig. 3. Light microscope images of lateral fields of *Steinernema* third-stage infective juveniles. A: *S. "glaseri"* NC 513; B: *S. "glaseri"* NC 513 (showing irregularities); C: *S. arenarium* (squashed specimen); D: *S. kushidai;* E: *S. kraussei;* F: *S. affine*.

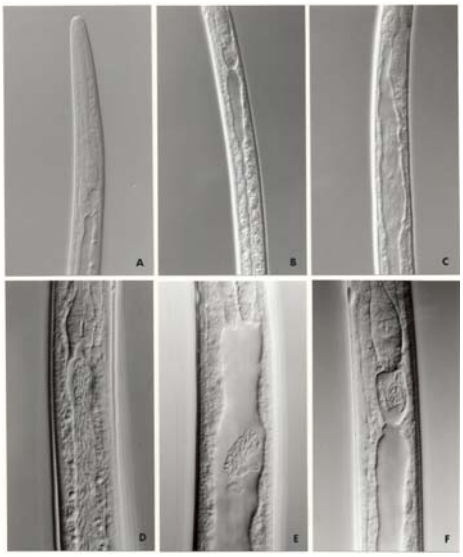

Fig. 4. Pharyngo-intestinal region of third-stage infective juveniles of *Steinernema arenarium* (A, B, E, F from Slovakia; D from France) and *S. "glaseri"* NC 513 (C). A: Pharynx region followed by intestine with wide lumen and anterior part slightly demarcated; B: Slightly offset anterior part of intestine, followed by a wide and empty intestinal lumen; C: Wide intestinal lumen with thread-like bacteria; D: Anterior region of intestine filled with bacteria; E: Aggregation of few bacteria in wide intestinal lumen; F: Bacteria in weakly demarcated part of intestine.

Tab. 1. Selected diagnostic characters of third-stage infective juveniles and males of species of the *Steinernema glaseri* group and other *Steinernema* species with long juveniles, each group in descending order of body length (for morphometrics range of means given and range of individual data in brackets).

Species	Infective juveniles					Males		
	Body length (µm)		D %		Lateral lines	D %		Mucron
glaseri	1130-1464	(726-1634)	65	(50-71)	9	70-91	(60-91)	-
cubanum	1124-1284	(975-1509)	66-70	(62-75)	9	70-75	(70-80)	-
puertoricense	1167-1171	(1050-1245)	66	(62-74)	9	77	(65-87)	-
ohioense	1160	(1075-1250)	61		?	?		?
arenarium	934-1232	(724-1580)	55-63	(52-68)	9	78-93	(52-102)	-
caudatum	1106	(933-1296)	53		9	71		-
longicaudum	960-1064	(900-1194)	55-57	(52-62)	9	64-75	(56-92)	-
loci	986	(896-1072)	57	(52-63)	9	73	(61-80)	-
karii	904-932	(832-982)	57	(53-61)	9	66-79	(53-89)	-
thanhi	851	(720-960)	58	(52-67)	9	73	(64-82)	-
oregonense	900-980	(820-1110)	50	(40-60)	7-9	70-73	(64-75)	-
neocurtille	885	(741-988)	12	(10-15)	5 (-9)	19	(13-26)	+
kraussei	820-951	(588-1200)	42-47	(38-52)	6+2	53-58	(52-68)	+
feltiae	632-880	(524-950)	44-48	(40-53)	7+2	60-62	(51-66)	+
sangi	753	(704-784)	40	(36-44)	5+4	49	(42-63)	±
bicornutum	664-770	(608-873)	44-50	(42-60)	7+2	50-55	(50-61)	+